Microsatellite Instability in Human Non-Melanoma and Melanoma Skin Cancer

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Microsatellite instability secondary to replication errors (RER), characterized by length changes at repetitive loci scattered throughout the genome, is a recently recognized genetic mechanism important in the development of some human cancers. Although RER has been reported in sebaceous gland tumors from patients with the Muir-Torre syndrome, the frequency of RER in human non-melanoma and melanoma skin cancers is not known. In this study, we investigated the importance of RER in human skin carcinogenesis. RER was identified in three of four actinic keratoses from a patient belonging to a kindred with documented Muir-Torre syndrome, which indicates that defective DNA replication may contribute to skin cancer development in such patients. Examination of a series of tumors from patients without Muir-Torre, including 137 skin cancers (47 basal cell carcinomas, 49 squamous cell carcinomas, and 41 primary malignant melanomas), 19 actinic keratoses, and 20 cases of Bowen's disease, using 10 or more microsatellite markers, identified repeat-sequence instability in less than 5% of the tumors studied. In six of the eight tumors, the sole change was an alteration 2 base pairs in length at a single locus. One patient with a squamous cell carcinoma showed changes at multiple loci suggesting defective mismatch repair. Although the low frequency of RER found in this study of a large series of human skin tumors suggests that this phenomenon is uncommon in patients with skin cancer, the identification of RER at multiple loci in two patients suggests that error-prone replication may be important in skin cancer development in some individuals. ***Key words: replication error/genetics/actinic keratosis. J Invest Dermatol 104:309–312, 1995***

Cancer is a genetic disease characterized by the preferential growth and selection of cells that have accumulated multiple mutations in both proto-oncogenes and tumor suppressor genes [1]. Epidemiologic analysis of the age-specific cancer incidence and molecular analysis of genetic changes in colorectal tumorigenesis have suggested that the development of most human epithelial cancers requires five or more independent genetic events [1]. Studies of the spontaneous mutation rate in normal human cells have led to the suggestion that a "mutator phenotype" is a prerequisite for cancer development in at least some tissues, as the normal mutation rate is only sufficient to account for two to three mutations in any given cell [2]. The increased rate of cancer development in inherited disorders such as xeroderma pigmentosum and Bloom's syndrome underscores the consequences of failure of the normal cellular mechanisms that maintain genomic integrity. To date, most studies of the genetic basis of human cancer, including skin cancer, have concentrated on the role of oncogenes and tumor suppressor genes in tumor development and progression [3–9]. The development of microsatellite markers for linkage analysis and the subsequent use of these markers to detect chromosome loss in tumor samples have led to the identification of a novel type of genetic alteration in some human tumors [10–17]. Microsatellites are short repetitive DNA sequences distributed throughout the genome. In contrast to the accurate replication of these sequences in normal cells, tumors from patients with hereditary non-polyposis colorectal cancer show alterations in repeat length at multiple loci, indicating microsatellite instability secondary to replication errors (RER) [10–12]. Linkage analysis of kindreds with hereditary non-polyposis colorectal cancer has established that the disease is genetically heterogeneous, and it is now known that the "mutator genes" on chromosome 2p and 3p21-23 responsible for this disorder are the human homologs of genes important in bacterial and yeast mismatch repair [18,19]. Studies of a variety of human cancers have suggested that the relative importance of replication error in tumor development varies among tumors arising in different organs [13,17].

Muir-Torre syndrome was originally characterized by the presence of sebaceous gland adenomas or carcinomas in association with an internal malignancy. However, other skin tumors including keratoacanthomas, basal cell carcinomas, squamous cell carcinomas (SCCs), actinic keratoses, and melanoma have been recorded in some patients with the disorder [20,21]. Muir-Torre syndrome is considered part of hereditary non-polyposis colorectal cancer/Lynch type II, in which germline mutations have been demonstrated in hMSH2 and hMLH1 genes [18,19,22]. Although RER has been reported in sebaceous gland tumors from patients with the Muir-Torre syndrome [20], the frequency of microsatellite instability in human melanoma and non-melanoma skin cancer is not known. In this study, we examined actinic keratoses, which are

Manuscript received August 27, 1994; revised November 18, 1994; accepted for publication November 25, 1994.

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Abbreviations: LOH, loss of heterozygosity; RER, microsatellite instability, secondary to replication errors.

0022-202X/95/$09.50 • SSDI0022-202X(94)00357-D •

dysplastic, potentially premalignant epidermal lesions, from a patient belonging to a kindred with the Muir-Torre syndrome to determine whether RER occurs in tumors other than sebaceous neoplasms in patients with this syndrome. Having established that this change can occur in other skin tumors, we went on to investigate the frequency of RER in a large series of sporadic non-melanoma and melanoma skin cancers.

MATERIALS AND METHODS

Tumor samples were studied from 47 basal cell carcinomas, 49 SCCs, 19 actinic keratoses, 20 cases of Bowen's disease, and 41 primary malignant melanomas. No patient was known to have Muir-Torre syndrome or to be from a kindred with Muir-Torre syndrome apart from patient 252. DNA from basal cell carcinomas and some SCCs was isolated from snap-frozen tumor tissue, as described previously [8]. DNA from the remaining tumor samples was isolated from microdissected, formalin-fixed archival material by proteinase K digestion and phenol-chloroform extraction [9]. Control DNA was isolated from matching normal skin and/or venous blood samples from these patients. Analysis for loss of heterozygosity (LOH) and RER was carried out by polymerase chain reaction amplification of microsatellite polymorphisms obtained from Research Genetics (Huntsville, AL), as described previously [9]. The following microsatellite markers were used: D1S201, D1S214, D1S212, D2S149, D2S163, D3S1293, D3S1268, D4S394, D4S402, D5S419, D5S410, D6S299, D6S262, D6S305, D6S281, D7S481, D7S495, D8S261, D8S257, D9S162, D9S171, D9S166, D9S175, D9S197, D9S160, D10S226, D10S185, D11S922, D11S910, D12S98, D12S86, D13S170, D13S155, D14S73, D15S118, D16S414, D16S422, D17S796, D17S785, D18S59, D18S70, D19S216, D19S225, D20S104, D20S100, D21S262, and D22S283. However, not all tumors were investigated at all these loci. Briefly, polymerase chain reaction was performed in 10-μl reactions using 100 ng of DNA, 200 μM dNTPs, 1 pmol of each primer (one end-labeled with γ^{32}P ATP), and 1 U of Taq DNA polymerase (BioTaq, Bioline, UK). Amplification consisted of 30 cycles of 1 min at 95°C, 1 min at 55°C, and 1 min at 72°C, with a final 10-min extension time at 72°C. For some primers, the annealing temperature was increased to 60°C to eliminate extra non-allelic bands. Ten microliters of loading buffer was added to each reaction, and the samples were heat denatured and electrophoresed through 6% denaturing polyacrylamide gels. Gels were dried and exposed to Fuji XR film for 1–24 h. A minimum of 10 loci were examined in each patient.

RESULTS

To determine whether RER occurs in skin tumors other than sebaceous tumors in patients with familial cancer syndromes associated with RER, we obtained samples from four actinic keratoses from a patient belonging to a previously described kindred with the Muir-Torre syndrome [23]. This fair-skinned individual presented in her mid-60s with multiple actinic keratoses. Notably, the patient did not have any sebaceous gland tumors and was otherwise well; she had no history of internal malignancy. Analysis of several microsatellite loci revealed microsatellite length alterations at multiple loci in three of the four actinic keratoses, indicating RER at these loci [10–12] **(Fig 1)**. The new bands detected in this patient were 2–24 base pairs larger or smaller than the allelic bands. No LOH was identified in these actinic keratoses at any locus; however, the multiple band shifts present at several loci made interpretation of LOH at these loci difficult.

The frequency of RER was then investigated in a large series of human skin cancers. Eight of the 176 tumors examined showed evidence of RER with altered electrophoretic mobility of microsatellite repeat fragments **(Table I)**. The observed band shifts produced new fragments larger and smaller than the normal alleles. One or both normal alleles was always seen in addition to the new fragments. In these patients without Muir-Torre syndrome, all eight tumors with RER showed LOH at other microsatellite markers, but there was no relation between the loci where LOH was identified and the loci where the band shifts were detected. Patient 193 demonstrated minor band shifts (2 base pairs) at some loci in association with apparent LOH of one of the normal alleles **(Fig 2)**. In six of the eight tumors, the sole change was a 2-base pair alteration in microsatellite length at a single locus. In one patient (no. 193), mobility shifts were observed at multiple loci, and the length changes at some loci were significantly greater than those

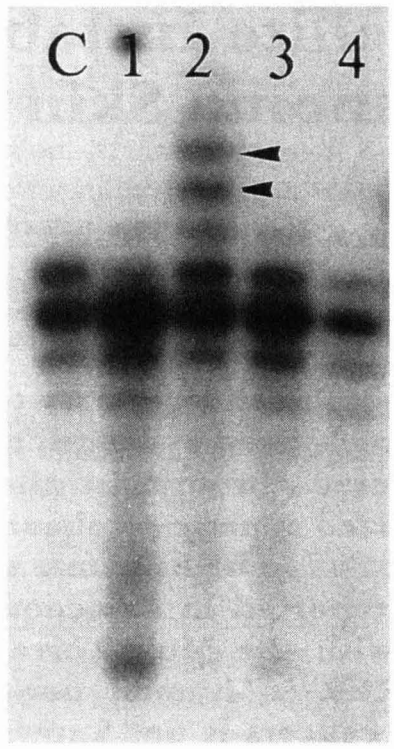

Figure 1. Microsatellite instability in actinic keratoses from a patient with the Muir-Torre syndrome. New bands at the D17S796 locus are due to alterations in repeat-sequence length in DNA from one of the actinic keratoses examined (*sample 2, arrowheads*). Actinic keratoses are from the left arm (*lanes 1, 2, 4*) and left leg (*lane 3*). C, control.

observed in other tumors **(Table I)**. In patient 219, RER was seen with two chromosome-9 microsatellite markers. In one patient (10275/92) with multiple actinic keratoses, a 2-base pair band shift was observed at the same locus in two separate lesions.

DISCUSSION

In this study, we investigated the importance of genomic instability as evidenced by microsatellite electrophoretic mobility shifts in a large series of human skin cancers. The finding of RER in actinic keratoses in a patient from a family with the Muir-Torre syndrome indicates that skin tumors other than sebaceous neoplasms show the same genetic abnormality as other internal malignancies in affected individuals with this syndrome [20]. Despite the absence of the classic clinical phenotype, there is strong circumstantial evidence that this patient has the underlying defect responsible for Muir-Torre: 1) The patient is from a typical kindred with Muir-Torre syndrome, in which sebaceous neoplasia and colonic carcinoma have been documented [23]; 2) there are large band shifts at multiple loci, characteristic of Muir-Torre/Lynch type II syndrome [10–15,20], in actinic keratoses from this patient; and 3) expressivity (rather than penetrance) of Lynch type II is known to be highly variable. Although the incidence of other skin tumors including basal cell carcinomas, SCCs, and actinic keratoses is greater in kindreds with the Muir-Torre syndrome [21], the high prevalence of non-melanoma skin cancer and the large number of people with undiagnosed non-melanoma skin cancers make it difficult to determine whether the increased incidence in these patients is real or is due to ascertainment bias. The identification of RER in three of four actinic keratoses from a patient belonging to a Muir-Torre kindred suggests that the defect in DNA replication in this disorder may contribute to skin cancer development in such patients.

In contrast to the high frequency of RER in sporadic colorectal, endometrial, and gastric cancers [10–17], changes in microsatellite length are uncommon in melanoma and non-melanoma skin cancers, occurring in less than 5% of the tumors examined. No significant difference was observed in the frequency of instability among the different tumor types. In their original description of RER in cancers of the proximal colon, Thibodeau *et al* [12] observed an inverse relation between LOH and RER. However, the coexistence of LOH and RER at different loci in all of the eight

Table I. Microsatellite Length Changes are Present at One or More Loci in Some Human Non-Melanoma and Melanoma Skin Tumors

Patient Number	Age (y)	Sex	Site of Lesion	Tumor Type	Number of Loci Altered/ Examined	Names of Altered Loci	LOH
219	78	M	Face	BCC	2/40	D9S165(9p), D9S197(9q)	D9S166(9p), D9S175(9p), D9S197(9q)
193	81	M	Hane	SCC	17/43	D1S201(1p), D3S1293(3p), D3S1268(3q), D6S299(6p), D6S262(6q), D6S281(6q), D7S495(7q), D8S257(8q), D11S910(11q), D12S86(12q), D13S170(13q), D17S796(17p), D18S59(18p), D18S70(18q), D20S100(20q), D21S262(21q), D22S283(22q)	D1S201(1p), D2S163(2q), D3S1293(3p), D3S1268(3q), D5S410(5q), D9S162(9p), D9S160(9q), D11S922(11p), D12S86(12q), D13S170(13q), D17S796(17p), D18S59(18p), D18S70(18q), D20S100(20q)
323	81	F	Leg	SCC	1/42	D12S86(12q)	D9S162(9p)
12254/93	83	F	Leg	Bowen's	1/11	D3S1293(3p)	D9S162(9p)
252[a]	53	F	Arm	Actinic keratosis	10/12	D2S149(2p), D7S495(7q), D9S162(9p), D9S197(9q), D10S226(10p), D11S910(11q), D12S98(12p), D13S170(13q), D17S785(17q), D22S283(22q)	No
			Arm	Actinic keratosis	11/12	D2S149(2p), D7S495(7q), D9S162(9p), D9S197(9q), D10S226(10p), D11S910(11q), D12S98(12p), D13S170(13q), D17S796(17p), D17S785(17q), D22S283(22q)	No
			Leg	Actinic keratosis	9/12	D2S149(2p), D7S495(7q), D9S162(9p), D9S197(9q), D10S226(10p), D11S910(11q), D13S170(13q), D17S785(17q), D22S283(22q)	No
9285/93	70	M	Hand	Actinic keratosis	1/11	D17S796(17p)	D9S160(9q), D17S796(17p), D17S785(17q)
10275/92	83	F	Head	Actinic keratosis	1/11	D3S1268(3q)	D2S149(2p), D2S163(2q), D3S1293(3p), D11S910(11q), D13S170(13q), D17S785(17q), D22S283(22q)
			Face	Actinic keratosis	1/10	D3S1268(3q)	D3S1293(3p), D9S162(9p), D13S170(13q), D17S785(17q), D22S283(22q)
116	64	M	Back	Melanoma	1/10	D3S1293(3p)	D8S257(8q)

[a] Patient from kindred with Muir-Torre syndrome. No other patient was from a kindred with a cancer family syndrome or had documented underlying disease.

sporadic skin tumors from patients without Muir-Torre syndrome in this study is similar to the findings in a recent study of RER in bladder cancer [16], suggesting that the two genetic events are not mutually exclusive.

The changes observed in the SCC from patient 193 and in the actinic keratoses obtained from the patient with a known familial cancer syndrome are similar to those described in replication-error–positive colorectal tumors. Therefore, mismatch repair may have been defective in these tumors. In contrast to the other patient with RER at multiple loci, patient 193 had no family or personal history of cancer in other organs, and although the SCC in this case was poorly differentiated, several other SCCs that were equally poorly differentiated showed no evidence of RER. The presence of multiple RERs in this patient's SCC suggests either that he had a somatic event in both copies of a mutator/replication-error–repair gene, or that he has a germline mutation of one of these genes and required only one somatic event to knock out the second allele.

The significance of minor microsatellite length alterations at single loci, as we found in six tumors, is not known. Analysis of colorectal tumors with minor alterations in microsatellite length at a small number of loci showed them to be clinically indistinguishable from colorectal cancers without RER. By contrast, colorectal cancers with larger length alterations, frequently at multiple loci, are clinically and pathologically distinguishable from other colorectal tumors. In a recent study, Parsons *et al* [24] demonstrated a 100-fold increase in the mutation rate of $(CA)_n$ sequences in these tumors. Although band shifts at single loci in skin tumors may reflect RER, other mechanisms also may be important, such as chromosome reduplication of the region that encompasses the microsatellite marker [25]. The finding of single band alterations at the same locus on chromosome 3q in two actinic keratoses obtained from the same patient (case 10275/92) warrants further investigation using additional markers within this region to determine whether there has been a structural rearrangement within this region.

Although the low frequency of RER found in this study of a large series of human skin cancers shows that this phenomenon is uncommon in patients with skin cancer, the identification of RER at multiple loci in two patients suggests that error-prone replication may be important in skin cancer development in some individuals. Based on the arguments discussed above, it seems likely that patient 252 with multiple actinic keratoses has Muir-Torre syndrome; therefore, multiple actinic keratoses may be a presenting feature of this disorder. Given the high prevalence of actinic keratoses and non-melanoma skin cancers, this observation also suggests that analysis of skin tumors for RER could prove useful in the diagnosis of patients genetically predisposed to cancer development due to mismatch repair defects. Such an approach might be especially useful in patients from kindreds without known germline mutations. Further studies of individuals from families genetically predisposed to internal malignancies should take advantage of the high prevalence of non-melanoma skin cancer and actinic keratoses, the

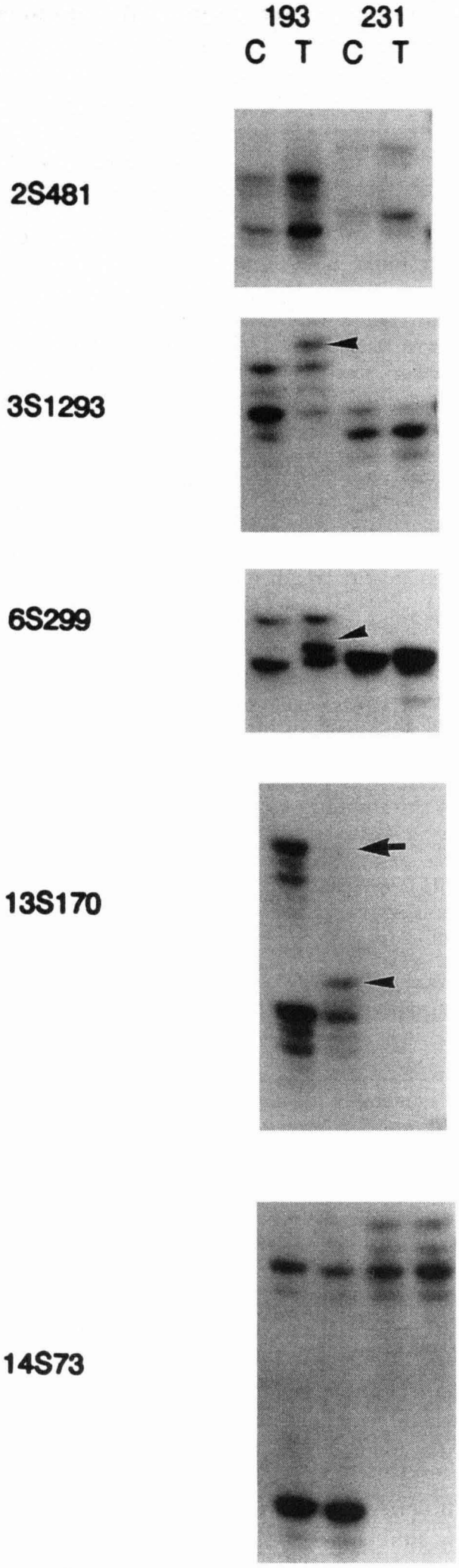

Figure 2. Microsatellite analysis at five loci in patients 193 and 231. Patient 193 shows alterations in repeat-sequence length at the D3S1293, D6S299, and D13S170 loci (*arrowheads*). This sample also shows LOH at the D13S170 locus (*arrow*). The microsatellite banding pattern at the other two loci is identical in tumor and normal DNA. No evidence of microsatellite instability is seen at any of the loci examined in patient 231. C, control; T, tumor (both examples are SCCs).

frequent occurrence of multiple primary tumors, and the availability of benign tumors with no malignant potential. Human skin is a powerful system for investigating the importance of RER in human tumor development.

AGQ and EH are MRC Training Fellows; IR is a North of England Cancer Research Campaign (NECRC) PhD student.

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